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Abstract

1. Submerged macrophytes are important foundation species that can strongly influence the structure and functioning of aquatic ecosystems, but only little is known about the temporal variation and the timescales of these effects (i.e. from hourly, daily, to monthly).
2. Here, we conducted an outdoor experiment in replicated mesocosms (1000 L) where we manipulated the presence and absence of macrophytes to investigate the temporal variability of their ecosystem effects. We measured several parameters (chlorophyll-a, phycocyanin, dissolved organic matter [DOM], and oxygen) with high-resolution sensors (15 min intervals) over several months (94 days from spring to fall), and estimated metabolic rates of each replicate ecosystem in a Bayesian framework.
3. Over the entire experiment, macrophytes had a negative effect on phytoplankton biomass, a positive effect on mean DOM concentration, and either a weak or no effect on mean metabolic rates, DOM composition, and conductivity. We also found that macrophytes increased the variance of DOC composition and metabolic rates, and, at some times of the year, increased the variance of phytoplankton biomass and conductivity. The observation that macrophytes decreased the mean but increased the variance of phytoplankton biomass is consistent with a model of competitive interactions between macrophytes and phytoplankton that we implemented here.
4. Our high-resolution time series embedded within a manipulative experiment reveal how a foundation species can affect ecosystem properties and processes that have characteristically different timescales of response to environmental variation. Specifically, our results show how macrophytes can affect short-term dynamics of algal biomass, while also affecting the seasonal buildup of DOM and the variance of ecosystem metabolism.

Introduction

Decades of research on submerged macrophytes have documented how these aquatic plants can influence a suite of ecosystem properties and processes (Carpenter & Lodge, 1986; Jeppesen *et al.*, 1997; Huss & Wehr, 2004; Reitsema, Meire & Schoelynck, 2018). Acting as foundation species (Dayton, 1972; Ellison *et al.*, 2005), macrophytes create and maintain habitats for other species, affect species interactions, and influence the dynamics of matter and energy in freshwater ecosystems (Carpenter & Lodge, 1986; Jeppesen *et al.*, 1997). Populations of individual macrophyte species, as well as species assemblages, can also influence how aquatic ecosystems respond to environmental change and the propensity of ecosystems to shift between alternative stable states in shallow lakes (Scheffer *et al.*, 1993; Blindow, Hargeby & Andersson, 1998; Faafeng & Mjelde, 1998). Importantly, while the net ecosystem effects of macrophytes in contrasting equilibrium states are well studied, much less is known about how macrophytes affect the temporal dynamics of ecosystem properties and processes over timescales ranging from hours, to days, to months (Mitchell & Rogers, 1985; Madsen & Adams, 1988; Iacarella *et al.*, 2018). High-resolution times series that capture both mean and variance responses of aquatic ecosystems are essential for predicting the effects of environmental change on aquatic ecosystems (Reitsema *et al.*, 2018; Hillebrand *et al.*, 2018) and improving their management in light of increasing disturbance and climate variability (Spears *et al.*, 2017).

The strong and persistent ecosystem effects of macrophyte communities are often linked to their competitive interactions with phytoplankton communities for dissolved nutrients and light (Carpenter & Lodge, 1986; Scheffer *et al.*, 1993). In shallow lakes, the positive feedback between light transmission and macrophyte biomass is an important reason why macrophytes help maintain a clear water state over a wide range of nutrient loading (Scheffer *et al.*, 1993, 2003; Blindow *et al.*, 1998). Many types of macrophytes are efficient

70 at taking up nutrients from the water and, if rooted, from the sediment, which can limit
71 phytoplankton growth at low to intermediate nutrient loading (Yamamichi *et al.*, 2018).
72 Furthermore, macrophytes can reduce fish predation pressure on the zooplankton
73 communities that graze on phytoplankton (Jeppesen *et al.*, 1997), and can also produce
74 allelopathic chemicals that inhibit phytoplankton growth (Gross, 2003; Hilt & Gross, 2008;
75 Nakai *et al.*, 2012). While it is known that such mechanisms can contribute to the positive
76 feedbacks that help maintain lakes in a clear water state, (Kéfi, Holmgren & Scheffer, 2016;
77 Iacarella *et al.*, 2018) surprisingly little is known about the seasonal dynamics of these
78 interactions, partly because (Carpenter, 1988; Benedetti-Cecchi, 2003) to capture variability
79 of phytoplankton communities over time and concurrently with other ecosystem properties.
80 This is a problematic knowledge gap because the variance of ecosystem properties is
81 increasingly recognized as an important dimension of overall ecosystem resilience
82 (Cottingham & Carpenter, 1998; Benedetti-Cecchi, 2003; Scheffer *et al.*, 2009; Vasseur *et*
83 *al.*, 2014; Zelnik, Arnoldi & Loreau, 2018).

84 In addition to the effects on phytoplankton dynamics, macrophytes are known to
85 affect the amount and composition of dissolved organic dissolved organic matter (DOM)
86 (Bolan *et al.*, 2011; Kellerman *et al.*, 2015), which is a diverse mixture of low and high
87 molecular weight components of different structure and composition (Bolan *et al.*, 2011;
88 Kellerman *et al.*, 2015). In the clear water state, phototrophic organisms such as macrophytes,
89 phytoplankton and bacteria produce low weight dissolved organic carbon (DOC) compounds
90 such as carbohydrates that are byproducts of photosynthesis (Carpenter & Lodge, 1986;
91 Retamal *et al.*, 2007; Bolan *et al.*, 2011; Reitsema *et al.*, 2018). Macrophytes can both
92 directly produce DOC, and indirectly reduce it by stimulating higher rates of DOC
93 degradation from epiphytic bacteria (Catalán, Obrador & Pretus, 2014). Given the importance
94 of interactions between macrophytes and different compositions of DOM in aquatic

ecosystems (Reitsema *et al.*, 2018)it is important to experimentally test how macrophytes can simultaneously affect the mean and variance of DOM concentration and composition (Findlay & Sinsabaugh, 2003; Catalán *et al.*, 2014; Reitsema *et al.*, 2018), and to consider such effects in models of ecosystem resilience to nutrient perturbation (Kéfi *et al.*, 2016; Spears *et al.*, 2017).

DOM dynamics driven by competitive interactions between macrophytes and phytoplankton can also alter ecosystem metabolism (Mitchell, 1989; Kaenel, Buehrer & Uehlinger, 2000; Findlay & Sinsabaugh, 2003; Reitsema *et al.*, 2018). Growth and decay of macrophyte tissue can strongly affect metabolic rates of shallow lakes, depending on plant density, diversity and lake depth (Żbikowski *et al.*, 2019). In shallow lakes with a given nutrient load, ecosystem productivity is typically higher when macrophytes are dominant over phytoplankton (Wetzel, 1964; Carpenter & Lodge, 1986; Brothers *et al.*, 2013). Macrophytes are known to be efficient photosynthesizers (Kaenel *et al.*, 2000), but also provide additional substrate for the growth of autotrophic periphyton and bacteria (Wetzel & Søndergaard, 1998; Brothers *et al.*, 2013). Additionally, the effects of macrophytes on the dynamics of DOC accumulation and decomposition can affect shifts between net autotrophy and net heterotrophy (Mitchell & Rogers, 1985; Madsen & Adams, 1988; Nielsen *et al.*, 2013). Overall, the potential effects of interactions between macrophytes and phytoplankton on whole ecosystem metabolism are increasingly well documented. However, the ability of macrophytes to resist or moderate perturbations to ecosystem metabolism in the context of global change depends on the relative importance of the described mechanisms and the temporal scale on which they each occur (Zelnik *et al.*, 2018). To our knowledge, only a few studies have investigated the effects of competition for light and nutrients between macrophytes and phytoplankton on dynamics of DOC and metabolism at the temporal

resolution necessary to understand how they interact (Benedetti-Cecchi, 2003; Zelnik *et al.*, 2018).

Here, we experimentally tested how macrophytes affect the temporal dynamics of oligotrophic aquatic ecosystems in 1000L mesocosms over an entire growing season. We manipulated the presence and absence of a macrophyte assemblage consisting of two common species, *Myriophyllum spicatum* and *Chara tomentosa,* and quantified several biotic (two phytoplankton pigments) and abiotic (temperature and conductivity, dissolved oxygen, dissolved organic matter) properties at high temporal resolution (15 min). We used this data set to test three hypotheses. First, we predicted that macrophytes would be able to suppress phytoplankton biomass across seasonal variation in light and temperature. Second, we predicted that macrophytes would increase overall rates of ecosystem metabolism because they are known to be efficient photosynthesizers. Third, we predicted that macrophytes would impact not only mean ecosystem properties such as phytoplankton biomass, DOM, and metabolism, but also their temporal variance in response to continual changes in resource availability. For this last hypothesis, we also tested whether we could generate observed contrasts in variability using a simple model of competitive interactions between phytoplankton and macrophytes. We compare our findings with previous empirical work and discuss the broad functional spectrum of macrophytes as foundation species in shallow lake ecosystems.

Material and methods

Experimental design and setup

In an outdoor mesocosm experiment, we manipulated the presence or absence of an assemblage of macrophytes including *Myriophyllum spicatum* (hereafter *Myriophyllum*), a

142 perennial vascular plant that grows vertically towards the water surface forming a canopy,
143 and *Chara tomentosa* (hereafter *Chara*), a green algae that forms tufts of calcium carbonate
144 encrusted stems (typically <30cm) on the sediment surface. We chose this assemblage
145 because both species are common in Europe and other parts of the world, they commonly
146 occur together in macrophyte assemblages, and their strong influence on lake ecosystems has
147 been previously documented (Van den Berg *et al.*, 1998; Ibelings *et al.*, 2007; Hilt & Gross,
148 2008; Nakai *et al.*, 2012).

149 We set up the experiment on a site next to Eawag Kastanienbaum (eight tanks total)
150 with four pairs of 1000L mesocosms (1 x 1 x 1 m), with each pair consisting of a mesocosm
151 with (M+) and without (M-) a macrophyte assemblage (Fig. 1). To prepare the mesocosms,
152 we first established a 2 cm thick layer of limestone gravel from a local quarry (2-4 mm grain
153 size) and a 1 cm thick layer of fine, oligotrophic sediment (Fiskal *et al.*, 2019) that we
154 collected from Lake Lucerne. Afterwards the mesocosms were filled with water from Lake
155 Lucerne, an oligotrophic lake (Fiskal *et al.*, 2019), and left for two weeks to allow the
156 sediment to settle and the mesocosm community to assemble. On May 25th, 2015, we
157 collected *Myriophyllum* from a stream in Oberriet (47°19'55.5"N 9°34'43.9"E), and kept the
158 plants overnight in additional outdoor mesocosms onsite. The following day we collected
159 *Chara* from Lake Lucerne (47°00'06.8"N 8°20'02.7"E) and planted both species in the
160 mesocosms. We then divided all the macrophyte material manually (on a large and moist
161 plastic sheet) into 18 similar sized portions based on either an equal number of shoots (i.e. for
162 *Myriophyllum),* or similarly sized tufts (i.e. for *Chara*). We used 10 portions to quantify the
163 initial plant biomass (cleaned of sediment, infauna removed, biomass dried for 48 hours at
164 45°C), and added 4 portions to the M+ tanks. To inoculate the M- mesocosms with
165 macrophyte associated invertebrate and bacterial communities, we submerged the remaining
166 four portions of macrophytes in large mesh enclosures in the middle of the water column for

two weeks. On July 4th, we added 20 µg/L of P and 144.7 µg/L of N (i.e. Redfield ratio) to every mesocosm to supplement the Lake Lucerne source water with nutrients. Over the course of the experiment we measured dissolved nutrient concentrations in the mesocosms on four occasions (July 15, Aug. 5, Sept 8. and Oct 20, Fig. S1). At the end of the experiment (Oct 23rd) we quantified total macrophyte biomass in terms of above-ground dry weight (procedure: see above). This included both the original inoculated species and a filamentous algae species that colonized the sediment surface of all the mesocosms (see Table S1).

Ecosystem dynamics measurement using multiparameter sondes

We measured high-frequency ecosystem dynamics in the mesocosms from July 18th to Oct 23rd, 2015, using four autonomous multi parameter instruments (EXO2 modular sensor platform [YSI-WTW], hereafter referred to as sondes). The sondes were placed approximately at the center of the mesocosm (~0.5 m depth), away from the walls and outside of patches of macrophytes. Additionally, we measured photosynthetically active radiation (PAR) in 15 min intervals using a quantum sensor (Li-Cor) installed onsite to estimate surface light irradiance. PAR and sonde temperature data (Fig. S2) were used together with the dissolved oxygen data to estimate metabolic rates (see *Ecosystem Metabolism Modelling*).

Sensors - The sondes were equipped with modular sensors that recorded the following ecosystem parameters at 15 minute intervals (see Table 1 for details): temperature, chlorophyll-a and phycocyanin (as proxies for phytoplankton biomass), dissolved oxygen, fluorescent dissolved organic matter (fDOM) and specific conductivity. The sondes were equipped with an autonomous wiper that cleaned the sensor heads once every hour. All sensors were thoroughly cleaned whenever the sondes were moved to another mesocosm (see *Contrasts and sampling design*).

Calibration - Prior to the experiment, we performed a 48h cross-comparison trial where we installed all the sondes in a single mesocosm, enabling us to correct for differences among sensors and calibrate them against each other. During the cross-comparison trial we also quantified chlorophyll-a concentration by analyzing water samples with high performance liquid chromatography (HPLC, Jasco), and calibrated the optical sensors installed on the sondes in accordance with the manufacturer's manual (YSI-WTW). Hence, we report chlorophyll-a as µg/L, Phycocyanin and fDOM as raw fluorescence units. The oxygen sensors were calibrated against water-saturated air.

Contrasts and sampling design - At the beginning of the experiment, all four sondes were randomly assigned to two pairs of M+ and M- tanks. Because we only had four sondes available, the four sondes were taken out of these tanks after 10 days, thoroughly cleaned, and then introduced to the two remaining pairs, where they were left for another 10-day period (Fig. 1). Over the entire study we repeated this two-part cycle five times, yielding five distinct periods in which all tanks were sampled (Fig. 2-4: t1-t5). On the third sampling period (t3) we reduced the length of the measurement period to 7 days per set of tanks due to battery issues with the Sondes. Between all transfers, we thoroughly cleaned the sondes by hosing down the sondes and sensor bodies with a power washer before reinstalling them. We included the distinct periods (t1-t5) resulting from the rotation scheme and each individual tank as a random effect in all statistical models (see *Statistical Analysis*).

Ecosystem metabolism estimation

We used the temperature and oxygen measurements (mg/L) from the sondes and the PAR-measurements from the light sensor to model whole ecosystem metabolic rates of each mesocosm (for an overview of the abiotic conditions see Fig. S2). We used the *streamMetabolizer* package (Appling *et al.*, 2018) in the programming language R (R Core

214 Team 2017), which applies inverse modelling to estimate daily rates of gross primary
215 productivity (P), respiration (R) and gas exchange (K600) as g O2/m^2/day. For every
216 modelled rate we calculated the ratio of P and R. Prior to modelling we smoothed all input
217 data with a 12-hour moving average window to facilitate model convergence (A. Appling,
218 personal communication) and for more conservative estimates. We used a Bayes-type model
219 with pooled K600 for gas-exchange and lognormal priors (K = 0-1). Because the dissolved
220 oxygen time series reflects oxygen produced and consumed by all organisms in the whole
221 ecosystem, we assumed the model reflects the net effects of any biomass changes throughout
222 the experiment, for example, due to plant or epiphytic growth, or biomass decay.

223 **DOC sampling**

224 For each pair of tanks within each measurement period (i.e. every 10 or 7 days: Table S2) we
225 took a water sample for the analysis of DOC concentration and absorbance properties (Fig.
226 S3). Water samples were filtered through 47mm ashed GF/Fs (6 hours at 450°C), acidified
227 with HCl 2 M and preserved at 4°C in the dark until analysis via high temperature catalytic
228 oxidation (TOC-VCS, Shimadzu), with a detection limit of 0.5 mg/L (±0.5). Specific
229 ultraviolet absorbances (SUVA) were measured on the same samples from scans (1 nm
230 intervals) on a Shimadzu UV1700 spectrophotometer, using 1 cm quartz cuvettes. We
231 selected absorbance at 254 nm ($SUVA_{254}$) as a proxy of aromaticity and reactivity of DOC
232 (Weishaar *et al.*, 2003). Furthermore, we measured $SUVA_{350}$, which is an indicator for how
233 much UVA radiation is absorbed in the water (Fischer et al., 2015). We normalized the
234 SUVA measurements by dividing the sample absorbances by the total DOC concentration
235 (Hansen et al., 2016). Finally, we calculated spectral slope ratio (SSR) as the ratio of linear
236 regressions of the log-transformed spectra of 275–295 nm and 350–400 nm (Helms *et al.*,
237 2008; Hansen *et al.*, 2016). SSR is a common proxy for DOC molecular weight, to which it

238 should be inversely related. We were unable to analyze two DOC timepoints over the course
239 of the experiment (Oct 2nd, and 17th) due to technical problems with the TOC analyzer.

240 **A model for competition between macrophytes and phytoplankton**

241 We used an existing model for competition between macrophytes and phytoplankton
242 (Scheffer & Carpenter, 2003) to explore how mesocosm phytoplankton dynamics might
243 differ in the presence and absence of macrophytes. This model assumes standard features of
244 macrophyte-phytoplankton interactions and implicitly accounts for competition for light and
245 nutrients (Fig. 5). In the model, growth of macrophytes M and of phytoplankton P is
246 determined by a gain and a loss term following:

247

248 $$\frac{dP}{dt} = r_P \frac{n}{n+h_P} \frac{1}{1+\alpha_P P} P - l_P P + \sigma\varepsilon_P(t) \text{ (eq1a)}$$

249 $$\frac{dM}{dt} = r_M \frac{1}{1+\alpha_M M+bP} M - l_M M \ + \sigma\varepsilon_M(t) \text{ (eq1b)}$$

250 Phytoplankton grows with a maximum growth rate r_P that is limited by nutrients n in a
251 saturating function with half-saturation constant hp. Limitation of phytoplankton growth by
252 macrophytes comes through nutrient availability given by eq2:

253 $$n = \frac{Ntot}{1+q_M M+q_P P} \text{ (eq2)}$$

254 where *Ntot* is the total amount of nitrogen in the system and nutrients decrease in a nonlinear
255 way depending on the biomass of macrophytes and phytoplankton. Parameters q_M and q_P
256 determine the strength of the response in decreasing nutrients per biomass increase in
257 macrophytes and phytoplankton, respectively. Phytoplankton growth is also limited by light
258 due to self-shading scaled by α_P where $1/\alpha_P$ is the biomass of phytoplankton that makes the
259 maximum growth rate equal to half, whereas loss is determined by loss rate l_p., Macrophyte
260 maximum growth rate r_M is limited only due to competition for light (in contrast to
261 phytoplankton which is also limited by nutrients). In that case, light limitation is driven by

262 self-shading through parameter a_M and due to shading by phytoplankton by parameter b. Loss
263 is determined by loss rate l_M. In this simplified model formulation, we ignore some
264 potentially important interactions for which we had no empirical data, including nutrient
265 uptake by macrophytes from the sediment, and interactions between macrophytes and
266 periphyton biomass over time.

267 We used model parameters such that both macrophytes and phytoplankton were
268 equivalent in the rates of growth (r_P=r_M=0.5), mortality (l_P=l_M=0.05), and self-shading
269 (α_P=α_M=0.01). Instead, we modeled asymmetry between macrophytes and phytoplankton in
270 terms of light and nutrient limitation. Phytoplankton growth was limited by nutrients (h_P =
271 0.2), through macrophytes having a stronger impact on retaining the available nutrients in the
272 water column (*Ntot*) (q_M = 0.075 and q_P = 0.005). Macrophytes became light limited by
273 phytoplankton due to shading (b = 0.02). We set *Ntot*=3.2. This is a total nutrient level value
274 for which the model can give rise to 2 alternative states, one state with both macrophytes and
275 phytoplankton present (M+) and an alternative with phytoplankton but no macrophytes (M-).
276 These two states resemble our experimental setup. We simulated model dynamics at these
277 two contrasting states in the presence of environmental stochasticity εP(t), εM(t) (iid different
278 for macrophytes and phytoplankton) with strength σ (=0.5). We produced 200 simulated sets
279 of 1000 timepoints in length for each of the two states using the same sequence of stochastic
280 realizations for both states. In that way, differences in the recorded standard deviation and
281 coefficient of variation were independent of the stochasticity and only due to the stability of
282 the two states. The model was implemented in MATLAB R2016b (Mathworks) using Grind
283 v2 (https://www.sparcs-center.org/resources/dynamical-modelling-tools.html). Equilibria and
284 eigenvalues were estimated numerically, stochastic equations were solved with Euler-
285 Murayama integration using a 0.01 step.

Statistical analysis

Data treatment - Prior to the statistical analysis we removed incomplete days at the beginning and end of each measurement period (five time series: t1-t5). After this, each of the five time series had 864 data points (15 min interval = 96 data points per day = 9 days) for t1-3 and 576 data points (= 6 days) for t4 and t5. In a second step, we identified residuals of the detrended data that were outside 2.5 times the interquartile range as outliers and removed them from the data set. Finally, we used sliding windows with a size of 96 timepoints (= 1 day) to calculate time series of mean and cv, resulting in 768 data points for t1-t3 and 480 data points for t4-t5 (8 and 5 days, respectively).

Ecosystem dynamics - We analyzed time series of chlorophyll-a, phycocyanin, dissolved oxygen and fDOM separately for each of the five measurement periods to account for any variation due to the sonde-switching. To test for effects of macrophytes on the mean and variance of each parameter we implemented a series of generalized additive models (GAM) using the R-package *mgcv (Wood, 2004)*: one model per parameter (chlorophyll-a, Phycocyanin, fDOM, oxygen concentration, conductivity) per measurement period (t1-t5) per metric (mean or CV), resulting in a total of 50 separate GAMs. Each model used data from all eight tanks to test for differences in the mean or coefficient of variation (CV), with the presence or absence of macrophytes as the independent variable and tank and pair (see Fig. 1) as random effects. All GAMs included a term that accounted for first order autocorrelation and used penalized thin plate regression splines with automatic knot selection.

In addition to the GAMs we also calculated pairwise log response ratios (LRR) for macrophyte presence in all five periods for the high frequency measurements. To do so we divided vectors of mean and CV (coming either from the sliding window for the water parameters or from the daily estimates of metabolism) for M+ by the corresponding vector of

M- for each given pair of tanks. We then calculated the natural logarithm for these ratios for each measurement period and for each tank (for a summary of all response ratios see Fig. 6).

Ecosystem metabolism - To test for statistical differences in metabolic rates, we used the output from the ecosystem metabolism models, which were 8 or 5 consecutive days for t1-t3, and t4-t5, respectively (streamMetabolizer does not provide estimates for the final day in a time series). In a similar fashion as for the ecosystem dynamics, each model used data from all eight tanks within a measurement period to test for differences in P, R or P:R, using macrophyte presence as the independent variable and pair and tank as random effects. We calculated LRRs in the same way as described for the high frequency ecosystem dynamics. We used paired t-tests to test for differences in metabolism CV for each measurement period.

DOC - We used paired t-tests to test for differences in mean and CV of total DOC concentration, $SUVA_{254}$ and $SUVA_{350}$, and SSR between mesocosms with and without macrophytes. For each date (10 dates in total, see Table S2) we performed separate tests for all four metrics (n=8 tanks). We performed t-tests with the *stats* R-package (R Core Team 2017), and calculated pairwise LRRs for all DOC metrics (for a summary of all response ratios see Fig. 6).

Results

Macrophyte biomass and nutrients

The overall biomass of the macrophyte community changed over the course of the experiment, decreasing in the M+ treatment and increasing slightly in the M- treatment. At the end of the experiment substantially less *Chara* biomass was present in the M+ mesocosms than at the beginning (from 165.1 ± 21.65 g to 5.08 ± 7.6 g dry weight/mesocosm, mean ± se, Table S1), whereas *Myriophyllum* biomass increased threefold from 2.84 ± 0.54 g to 8.45 ±

1.6 g dry weight (mean ± se). In the M- treatment there was no *Myriophyllum*, but *Chara* biomass increased slightly due to growth from the sediment (from 0 to 0.27 ± 0.54 g dry weight, mean ± se). In both treatments, filamentous algae grew over the course of the experiment to a final biomass of 8.33 ± 10.54 g dry weight (M+) and 3.21 ± 5.46 g dry weight (M-, mean ± se). Throughout the experiment we observed no differences in concentrations of phosphate or nitrogen between mesocosms with and without macrophytes (Figure S1). The nutrients we supplied on July 4th were almost completely consumed by July 18th and were consistently low (<2ug P/L, <50ugN/L) over the entire experiment. However, concentrations of both nutrients tended to increase towards the end of the experiment, likely due to decomposition of plant material (e.g. *Chara*).

Ecosystem dynamics

As expected, solar radiation and water temperature decreased strongly over the course of the experiment from July 18th to Oct 20th (Fig. S2). Several parameters differed between M+ and M- tanks over the course of the experiment, with the magnitude of the difference depending on period (Fig 2 and Fig. 6; for P-values see Table 2). As expected, mean phytoplankton biomass was significantly higher without macrophytes (M-) in three of the five periods (t2, t4, and t5; Table 2), and, unexpectedly, the CV of phytoplankton biomass was higher in the tanks with macrophytes (M+) in three periods (t1, t2, and t5, Fig. 3). By comparison, mean phycocyanin was not significantly different between M+ and M- (Fig. 2), but the CV of phycocyanin was significantly higher in the M+ treatment during three periods (Fig. 3; t1, t2, t4). In tanks with macrophytes (M+), fDOM was higher in four periods (GAM, t2 - t5), and the CV was significantly lower in one period (GAM, t3). The mean concentration of dissolved oxygen was significantly higher in M+, but only towards the end of the experiment (Fig. 3, t4 and t5). In these two periods when irradiance was decreasing (Fig. S2),

357 the tanks lacking macrophytes (M-) became undersaturated with dissolved oxygen indicating
358 net heterotrophy. During the entire experiment, there were no differences between M+ and
359 M- in the CV of dissolved oxygen. Effect sizes of macrophyte presence on mean and variance
360 of all parameters measured in high frequency are summarized in Figure 6.

361 **Ecosystem metabolism**

362 We found weak and seasonally variable differences in mean ecosystem metabolism between
363 mesocosms with and without macrophytes (Fig. 4). In three measurement periods mesocosms
364 with macrophytes had significantly higher gross primary productivity (t1, t3, and t5). During
365 t1, mesocosms with macrophytes also had higher respiration (GAM, main effect of
366 macrophytes, $P = 0.001$). In t2 there was a tendency for higher P:R ratio in mesocosms
367 without macrophytes (GAM, main effect of macrophytes, $P=0.074$), but in t3 and t4 we found
368 the opposite pattern with significantly higher P:R ratio in the presence of macrophytes
369 (GAM, main effect of macrophytes, $P<0.001$ and $P=0.002$, respectively. Overall, P and R
370 decreased significantly over the course of the experiment, likely due to seasonal dynamics
371 (decreasing temperature and light, Fig. S2) but the P:R ratio remained around one. Across all
372 measurement periods, both productivity and respiration increased with chlorophyll-a biomass
373 (slope in Fig. S4). However, for a given chlorophyll-a concentration, both metabolic rates
374 were higher in the presence of macrophytes than in their absence (intercept in Fig. S4).
375 Moreover, we found higher variance of metabolic rates when macrophytes were present (all t-
376 tests of metabolism CV significantly different - Fig 6).

377 **DOC**

378 Total DOC concentration was not significantly different between M+ and M- mesocosms
379 (Table S2, Fig. S3). However, there were clear effects of macrophytes on chromophoric
380 (impacting light transparency) DOC components: $SUVA_{254}$ and $SUVA_{350}$ were often higher

in the presence of macrophytes (Table S2, Fig. S3), indicating that less UV light was able to penetrate in these ecosystems. SSR diverged among treatments early in the experiment and remained higher in the –M treatment for most of the season (Fig. S3), potentially indicating dissolved substances of lower molecular weight in the absence of macrophytes (e.g. sugars or amino acids). We also found higher variance in all metrics of DOC composition in the presence of macrophytes (Fig 6).

Simulated interactions between macrophytes and phytoplankton

Our simulation model produced results parallel to those observed in the mesocosms. Under identical nutrient levels, phytoplankton biomass was on average lower in the presence of macrophytes, but also varied more strongly around the mean (i.e. lower mean and higher CV under M-). This was also reflected in the stability regimes measured as the dominant eigenvalue lambda, which was higher in the absence and lower in the presence of macrophytes (Fig. 5, panel B). These results emerged solely from differences in the relative effects of macrophytes vs. phytoplankton on nutrient vs. light limitation and illustrate that differential competition for these resources can impact both mean and variance in phytoplankton biomass.

Discussion

Over the course of our experiment, macrophytes affected a wide range of ecosystem parameters. Most notably from those measured at high frequency, chlorophyll-a fluorescence (i.e. phytoplankton biomass) was significantly lower in the presence of macrophytes. This was expected, and in agreement with a large body of previous work documenting the outcome of competition between macrophytes and phytoplankton for dissolved nutrients and light (Sand-Jensen & Borum, 1991; Scheffer *et al.*, 1993; Faafeng & Mjelde, 1998; van Nes,

Rip & Scheffer, 2007). The ability of macrophytes to keep phytoplankton biomass low is important for stabilizing the clear water state in response to nutrient additions (Scheffer *et al.*, 1993; Ibelings *et al.*, 2007), and for understanding the timescale of competition for light and nutrients between these producers in the context of ecosystem stability. However, our high-resolution measurements also revealed some unexpected variance patterns of macrophyte-ecosystem interactions, most notably higher variance of phytoplankton and DOC components in the presence of macrophytes. While the former may be explained by resource competition between macrophytes and phytoplankton, as indicated by our competition simulation, the mechanisms behind elevated DOC variability are potentially related to growth and decomposition of macrophytes. Below we discuss the implications of our joint findings from the high-resolution time series and the simulation model, as well as the outcomes of the ecosystem metabolism models. Overall, our findings indicate that some macrophyte effects on ecosystem parameters are of more limited duration (e.g. phytoplankton was decreased only temporarily and most strongly in t2), whereas others remain stable across the season (e.g. fDOM was consistently higher from t2 onwards).

As expected from existing theoretical and experimental work, and confirming our first hypothesis, we observed higher phytoplankton biomass in the absence of macrophytes (Scheffer *et al.*, 1993; Blindow *et al.*, 1998). However, a finding we did not expect based on existing theory was the higher variability of phytoplankton biomass in the presence of macrophytes, a phenomenon that has not been previously reported. One mechanism for higher variability of phytoplankton biomass could be that the ongoing photosynthesis, growth, and decay of macrophytes increases the short-term variability of nutrient and carbon availability, and that phytoplankton respond more rapidly to these changes in nutrient concentrations than macrophytes themselves (Setaro & Melack, 1984; Mitchell, 1989; Eichel *et al.*, 2014). Importantly, however, the much larger reservoir of macrophytes biomass may

429 be able to repeatedly suppress these rapid increases in phytoplankton growth. Rooted
430 macrophytes build up biomass over time and can also store nutrients (Faafeng & Mjelde,
431 1998; Søndergaard & Moss, 1998; Yamamichi *et al.*, 2018), and thus probably prevented a
432 high mean level of phytoplankton biomass and repeatedly suppressed multiple bouts of
433 phytoplankton growth.

434 We implemented a model to explore how competitive interactions between
435 macrophytes and phytoplankton might affect the mean vs. the variance of phytoplankton
436 biomass. Specifically, we modelled competitive interactions such that macrophytes limit
437 nutrient availability and phytoplankton limit light availability (Scheffer and Carpenter
438 (2003)). This model reproduced the same contrast in phytoplankton biomass that we observed
439 in the mesocosms: lower mean phytoplankton biomass but higher variance (CV) in the
440 presence of macrophytes. Thus, the model predicted that phytoplankton biomass in a
441 phytoplankton-dominated state would be more stable than in a macrophyte-dominated state
442 under the same nutrient loading condition. At first sight, this result might appear
443 counterintuitive as a macrophyte-dominated state is expected to be more stable to the
444 unfavorable phytoplankton-dominated state. The biological explanation may be that when
445 macrophytes and phytoplankton are competing for nutrients (and light), variation arising from
446 the depletion of these resources is larger than with just one consumer (i.e. only phytoplankton
447 in M-). However, whether variability is always expected to be higher in a macrophyte
448 dominated than in a phytoplankton-dominated state, or under what conditions, would require
449 more empirical work to validate. The model shows that this is the case when considering only
450 one aspect of macrophyte-phytoplankton interactions (i.e. competition), which qualitatively
451 matched with the high-resolution algal biomass data we collected. However, macrophytes can
452 affect other compartments of the ecosystem (e.g. sediment, epiphytes, DOC) that are not
453 considered in our model. For example, macrophytes can produce allelochemicals that inhibit

454 phytoplankton production (Hilt & Gross, 2008; Nakai *et al.*, 2012), modify the light
455 environments via the production of DOC, or alter community structure of grazers; all of
456 which could potentially influence the variance of phytoplankton biomass. Nevertheless, our
457 study does illustrate that high resolution monitoring of ecosystem conditions (Mandal *et al.*,
458 2019) and accompanying simulation models may provide new insights into the underlying
459 mechanisms whereby macrophytes (or other foundation species) can affect ecosystem
460 dynamics in general, and the relationships between mean and variance of ecosystem
461 responses in particular.

462 In line with macrophytes being efficient primary producers in shallow lakes (Kaenel
463 *et al.*, 2000), we confirmed our second hypothesis that mesocosm ecosystems with
464 macrophytes had higher metabolic rates than those without macrophytes. Differences in
465 productivity were most pronounced in July, where mesocosms with macrophytes were
466 significantly more productive than macrophyte free mesocosms (t1). However, this difference
467 disappeared during the phytoplankton bloom in the second measurement period (t2). This
468 suggests that at intermediate concentrations, phytoplankton can increase productivity of
469 aquatic ecosystems and match rates of primary production of macrophytes. Yet for any given
470 chlorophyll-a biomass we measured, metabolic rates were higher when macrophytes were
471 also present. This indicates that even at relatively low density, macrophytes (*Myriophyllum*,
472 *Chara* and filamentous algae) can produce a significant metabolic signal. Higher productivity
473 of ecosystems with macrophytes was also reflected in P:R ratio, which is on average slightly
474 higher for those mesocosms in t3 and t4 (Sep 5th - Oct 9th). During t2 (Aug 7th - Aug 27th)
475 there was a tendency for higher P:R in mesocosms without macrophytes, probably due to
476 very high phytoplankton biomass. Towards the second half of the experiment, the growth of
477 filamentous algae may have also contributed to higher rates of whole ecosystem productivity
478 in +M tanks, where filamentous algae biomass was higher (8.33 ± 10.54 g dry weight, mean

479 ± SD) than in the -M tanks (3.21 ± 5.46 g dry weight, mean ± SD). Overall, these findings
480 suggest that macrophytes, regardless of their growth form, might make shallow lake
481 ecosystems more productive across the seasonal succession of ecosystem metabolism
482 (Madsen & Adams, 1988; Blindow *et al.*, 2006; Brothers *et al.*, 2013). These dynamics
483 require additional investigation, especially in the context of successive phytoplankton blooms
484 and their effects on the macrophyte community, but also in the context of rising temperatures
485 and eutrophication.

486 Another important axis by which macrophytes affected the experimental ecosystems
487 is their effects on the concentration and composition of dissolved organic matter. From the
488 beginning of t2 (August 8th) to the end of the experiment (October 23rd), fDOM
489 measurements in mesocosms with macrophytes were nearly twice as high as in mesocosms
490 without macrophytes. Higher mean, but also lower variance of DOM was expected, because
491 especially *Myriophyllum* is known to produce allelochemicals to inhibit algae growth that can
492 persist in the water column (Hilt & Gross, 2008; Nakai *et al.*, 2012). However, total DOC
493 concentrations were similar in both treatments, suggesting that not all components of the
494 DOM-pool are affected the same way by macrophytes (Catalán *et al.*, 2014; Reitsema *et al.*,
495 2018). Moreover, measurements from the scanning spectrophotometer showed consistently
496 lower SSRs, indicating the presence of DOC compounds with higher molecular weight. The
497 buildup and decay of macrophyte detritus could explain the low SSR ratios at similar total
498 DOC levels, particularly since much of the initial *Chara* biomass contributed to
499 decomposition rather than taking root, and/or grew but then decayed over the course of the
500 experiment. However, *Myriophyllum* biomass also increased substantially, and could have
501 added high MW compounds into the mesocosms. It is also possible that production rates of
502 DOC were similar in M+ and M- treatments (as the total DOC was similar), but that material
503 originating from macrophytes has a higher MW, and is more difficult to break down by

504 bacteria (Bolan *et al.*, 2011; Reitsema *et al.*, 2018). Overall, changes in DOC composition
505 and variance might reflect differences in the balance of production and decomposition rates
506 of different photosynthetic compounds, such as low MW sugars that are a byproduct of recent
507 photosynthetic activity (Carpenter & Lodge, 1986; Bolan *et al.*, 2011; Reitsema *et al.*, 2018).
508 However, more work needs to be done to understand the specific mechanisms behind such
509 patterns, e.g. biomass production and decomposition or the production of secondary
510 metabolites.

511 Using a common macrophyte assemblage, our experiment shows that communities of
512 submerged plants can affect the mean and variance of a wide range of biotic and abiotic
513 ecosystem properties and processes over a relatively short amount of time. Some of the
514 effects we found on mean values, such as macrophytes decreasing phytoplankton biomass
515 and increasing fDOM are not particularly surprising nor are they novel. However, the
516 elevated variability of both phytoplankton pigments in the presence of macrophytes was
517 unexpected, and potentially linked to competitive interactions. Across all our ecosystem
518 metrics, we found that changes in CV covaried negatively with changes in the mean, or that
519 CV increased despite no effect on the mean. Such results, show the importance of considering
520 also the variance of ecological dynamics, which is increasingly recognized as an important
521 aspect of ecosystem dynamics (Carpenter, 1988; Benedetti-Cecchi, 2003) and is used in a
522 wide array of applications, e.g. ecological forecasting (Petchey *et al.*, 2015; Pennekamp *et al.*,
523 2019), early warning signals for critical transitions (Scheffer *et al.*, 2009; Carpenter *et al.*,
524 2011), and ecological modelling (Bartell *et al.*, 1988; Cottingham & Carpenter, 1998).
525 Furthermore, our high frequency measurements can begin to reveal and quantify
526 characteristic differences in timescales of ecosystem change, such as the high variability in
527 phytoplankton communities vs. the relative stability of DOM and oxygen concentration
528 throughout the season. Future experiments targeting shallow lake ecosystems should also

encompass measurements in high resolution, e.g. to detect the potential outcome of interactions among different trophic levels (e.g. between macrophytes, zooplankton and fish) or quantify the response to perturbations (e.g. nutrients or temperature). Our study highlights how complex and temporally variable interactions around foundation species can be and underscores the need for further research that investigates biotic and abiotic components of these networks of interactions in detail.

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Data Availability Statement

Upon publication, all collected data will be made available via a data repository (Dryad).

Conflict of interest

The authors declare no conflict of interests.

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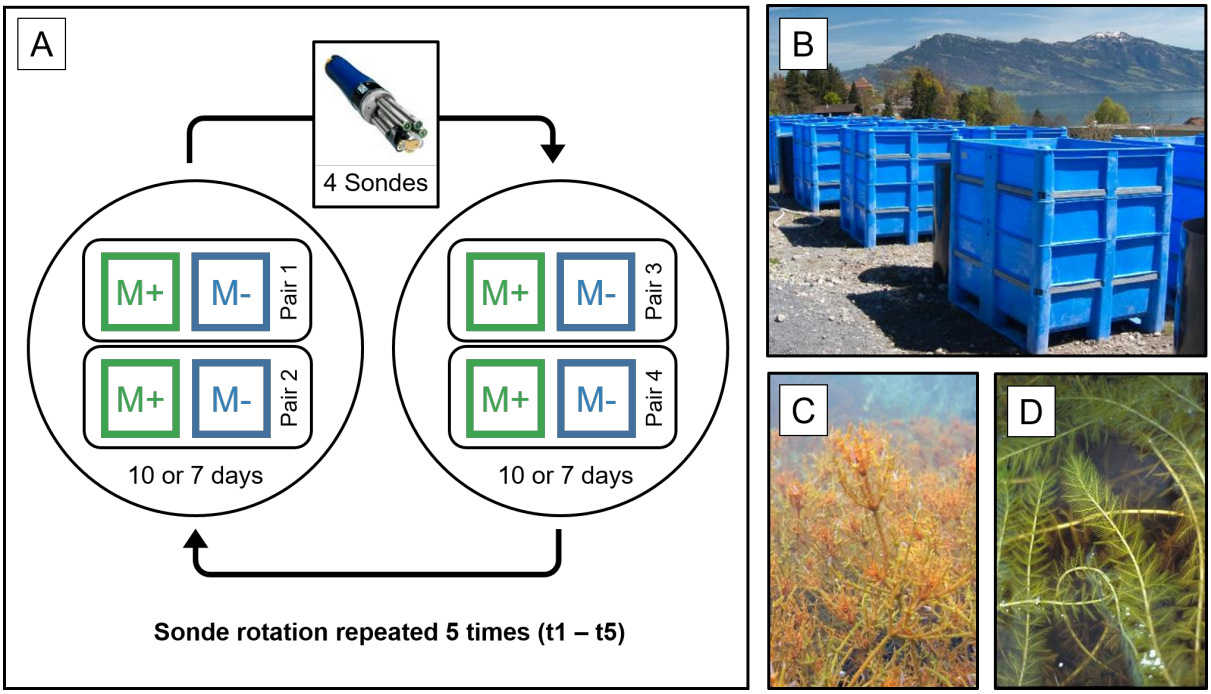
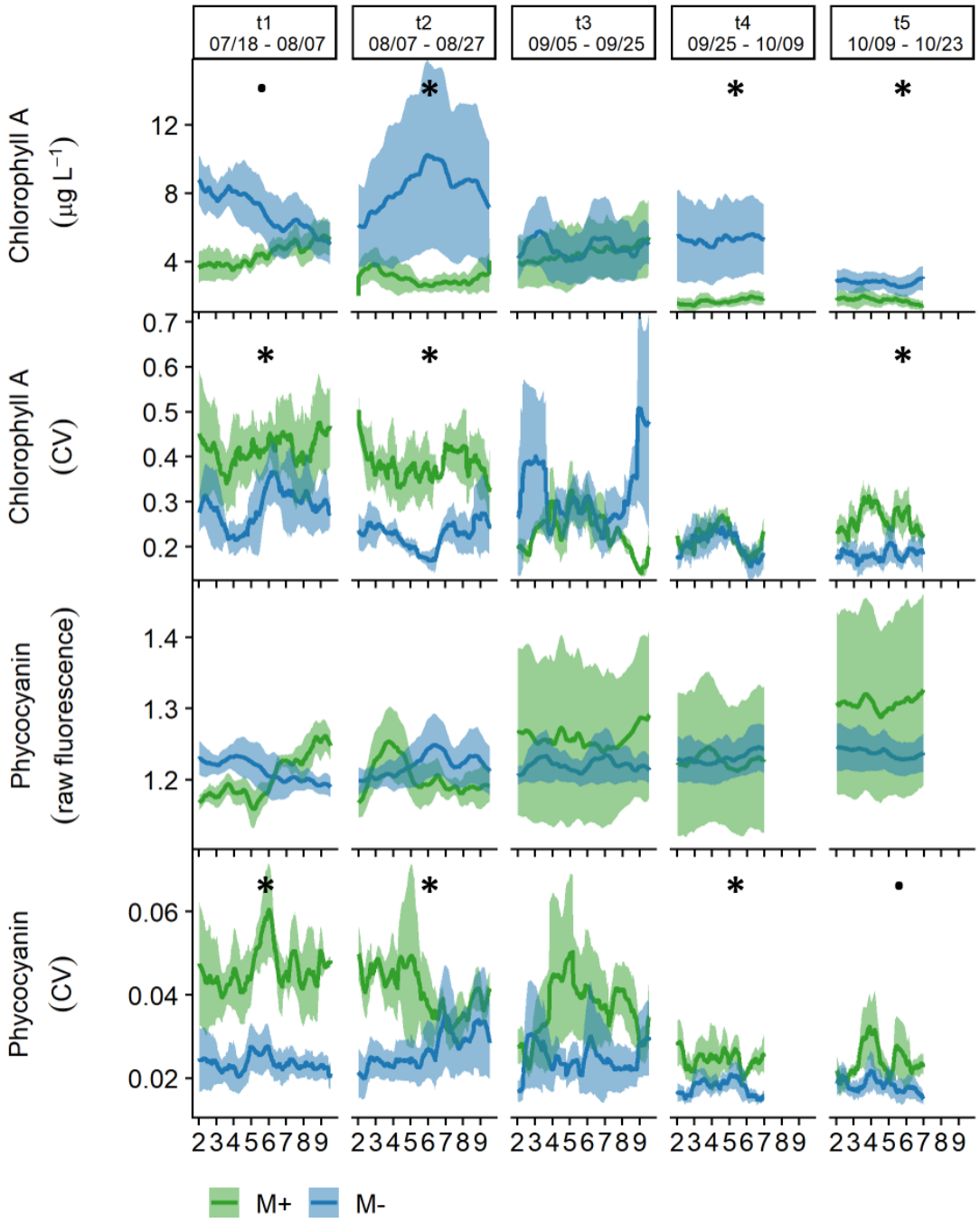
Figures

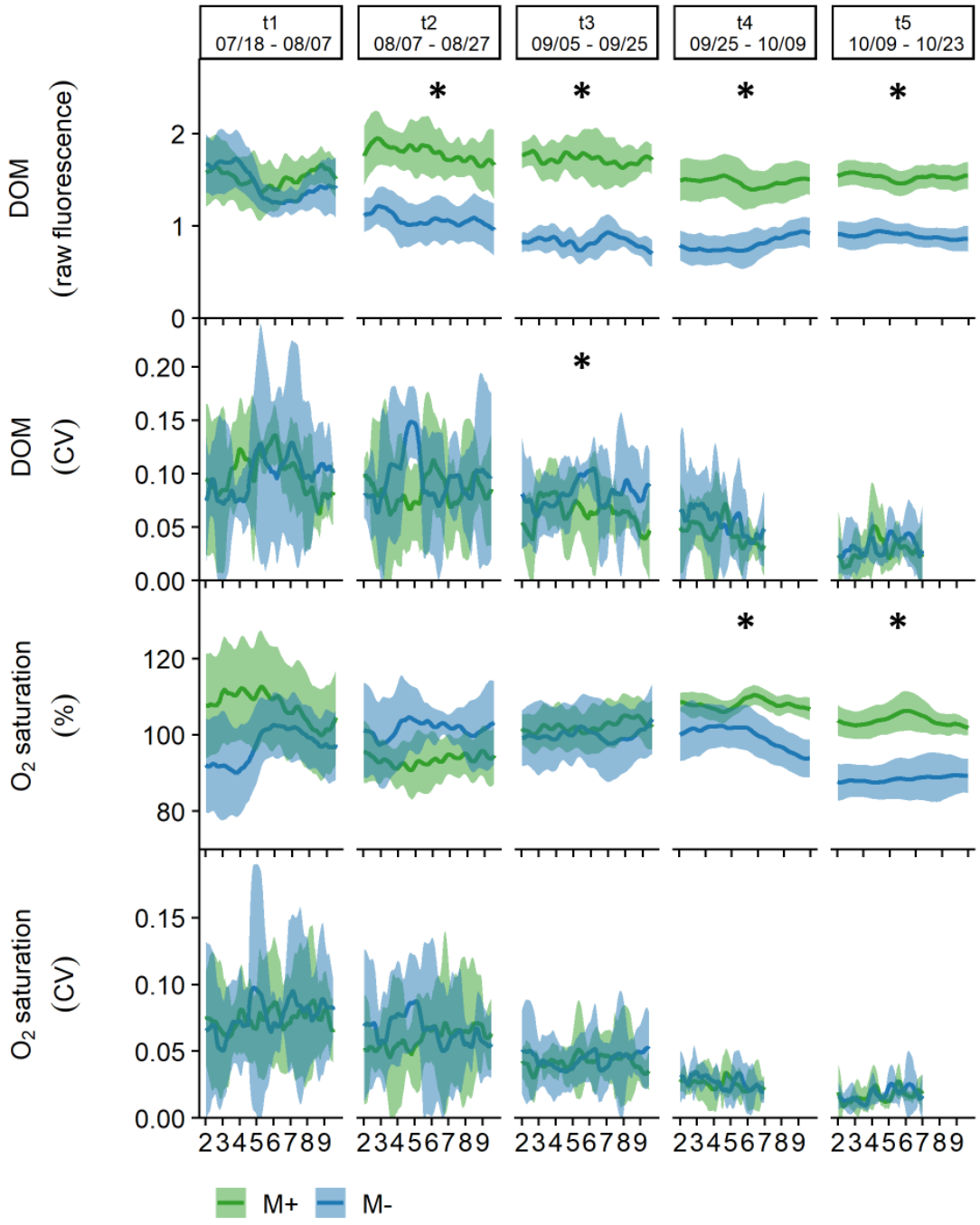


1. A: Scheme of experimental procedure. Because we were limited to four sondes, we could only measure two tank pairs of macrophyte (M+)/no macrophyte (M-) contrasts. To measure all eight tanks, we followed a rotation scheme in which every tank was measured for 10 consecutive days before the sondes were moved to another tank (for details refer to Methods section). B: Picture of experimental site showing the set up mesocosms (1000L). C: *Chara tomentosa* (Photo credit: Gustav Johansson). D: *Myriophyllum spicatum* (Photo credit: Alison Fox).


t1
07/18 - 08/07
t2
08/07 - 08/27
t3
09/05 - 09/25
t4
09/25 - 10/09
t5
10/09 - 10/23
Chlorophyll A
(μg L⁻¹)
Chlorophyll A
(CV)
Phycocyanin
(raw fluorescence)
Phycocyanin
(CV)
M+
M-

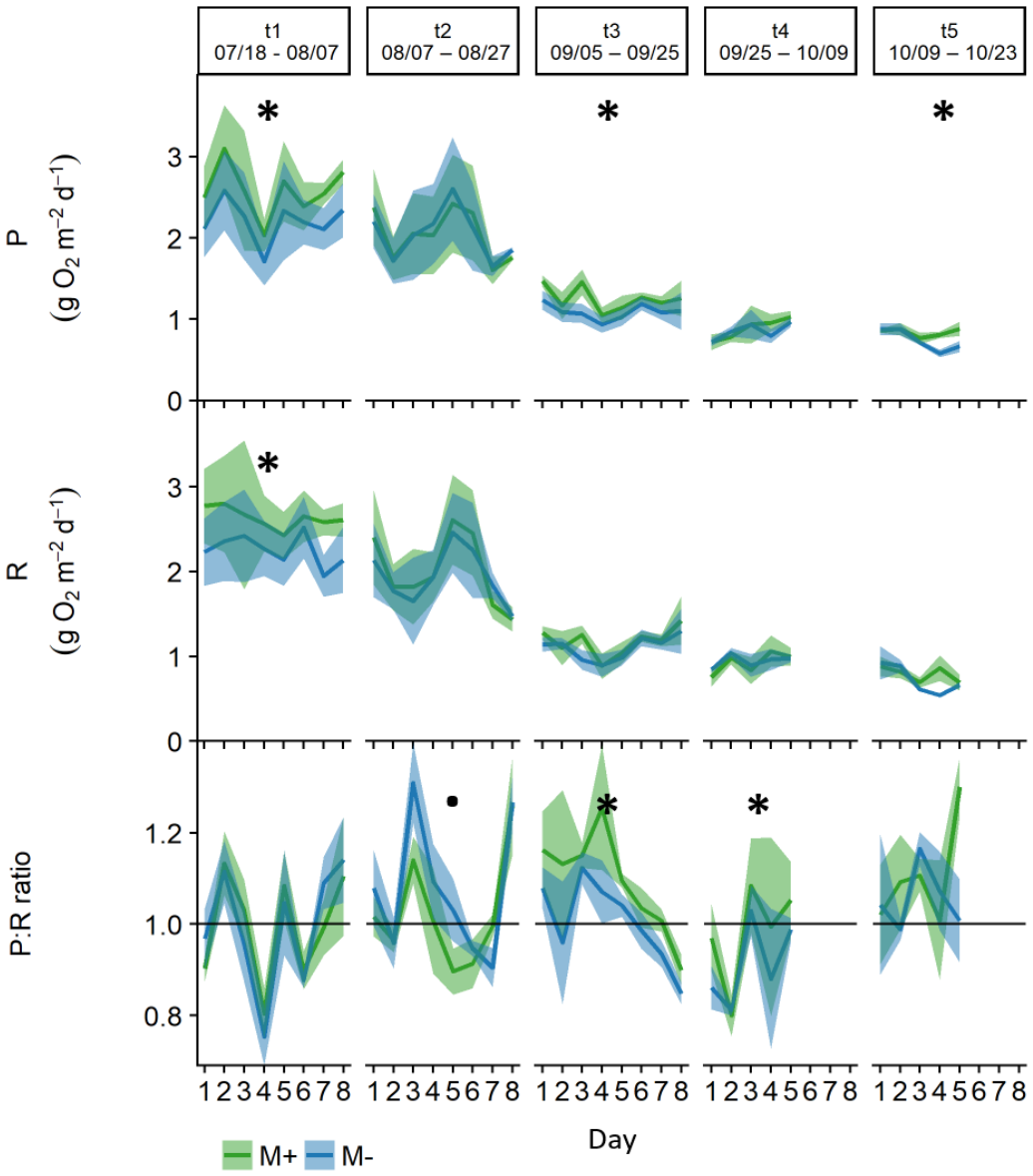
758

2. Sliding window results from high frequency measurements of chlorophyll-a and Phycocyanin over time (days 2-9 in each of five consecutive sampling periods). Lines show Mean ± SE (n = 8 tanks), asterisks indicate significant differences ($p <= 0.05$), dots indicate marginal significance ($p <= 0.1$). One GAM was used per period, including tank and the pair it was in (see Fig.1) as random effects. Here the sliding window time series of the Mean from both blocks are shown pooled for better illustration. Because the sliding window had a width of one day, only aggregate days 2-9 for each measurement are shown.


t1
07/18 - 08/07
t2
08/07 - 08/27
t3
09/05 - 09/25
t4
09/25 - 10/09
t5
10/09 - 10/23
DOM
(raw fluorescence)
DOM
(CV)
O2 saturation
(%)
O2 saturation
(CV)
M+
M-

766

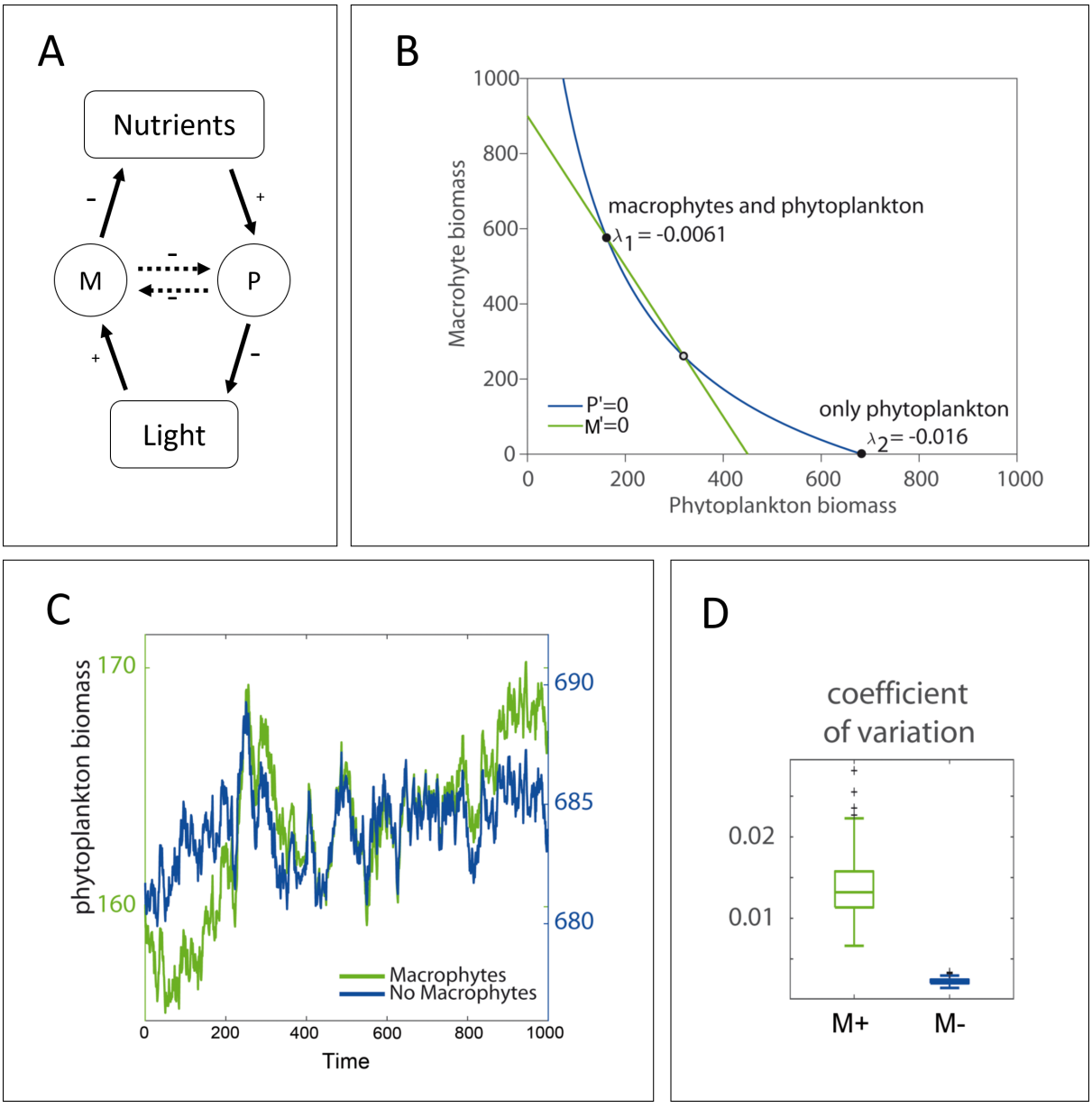
3. Sliding window results from high frequency measurements of fDOM and dissolved oxygen over time (days 2-9 in each of five consecutive sampling periods). Lines show Mean ± SE (n = 8 tanks), asterisks indicate significant differences ($p <= 0.05$). One GAM was used per period, including tank and the pair it was in (see Fig.1) as random effects. Here the sliding window time series of the Mean from both blocks are shown pooled for better illustration. Because the sliding window had a width of one day, only aggregate days 2-9 for each measurement are shown.


t1
07/18 - 08/07
t2
08/07 – 08/27
t3
09/05 – 09/25
t4
09/25 – 10/09
t5
10/09 – 10/23
P
(g O2 m−2 d−1)
R
(g O2 m−2 d−1)
P:R ratio
Day
M+
M-

774

4. Ecosystem productivity (P), respiration (R) and P:R ratio calculated from high frequency measurements of O2 saturation, temperature, light, and air pressure. Shown are Mean ± SE (n= 8 tanks), asterisks indicate significant differences, dots indicate marginal significance (p <= 0.1). One GAM was used per period, including both consecutive blocks as random variables. Here the time series of metabolic rates from both blocks are shown pooled for better illustration. The modelling procedure requires full days to be included, but because of the model parameterization to start each day 1 hour before sunrise, the last day is incomplete and thus cannot be modeled. Hence, only aggregate days 1-8 are shown.

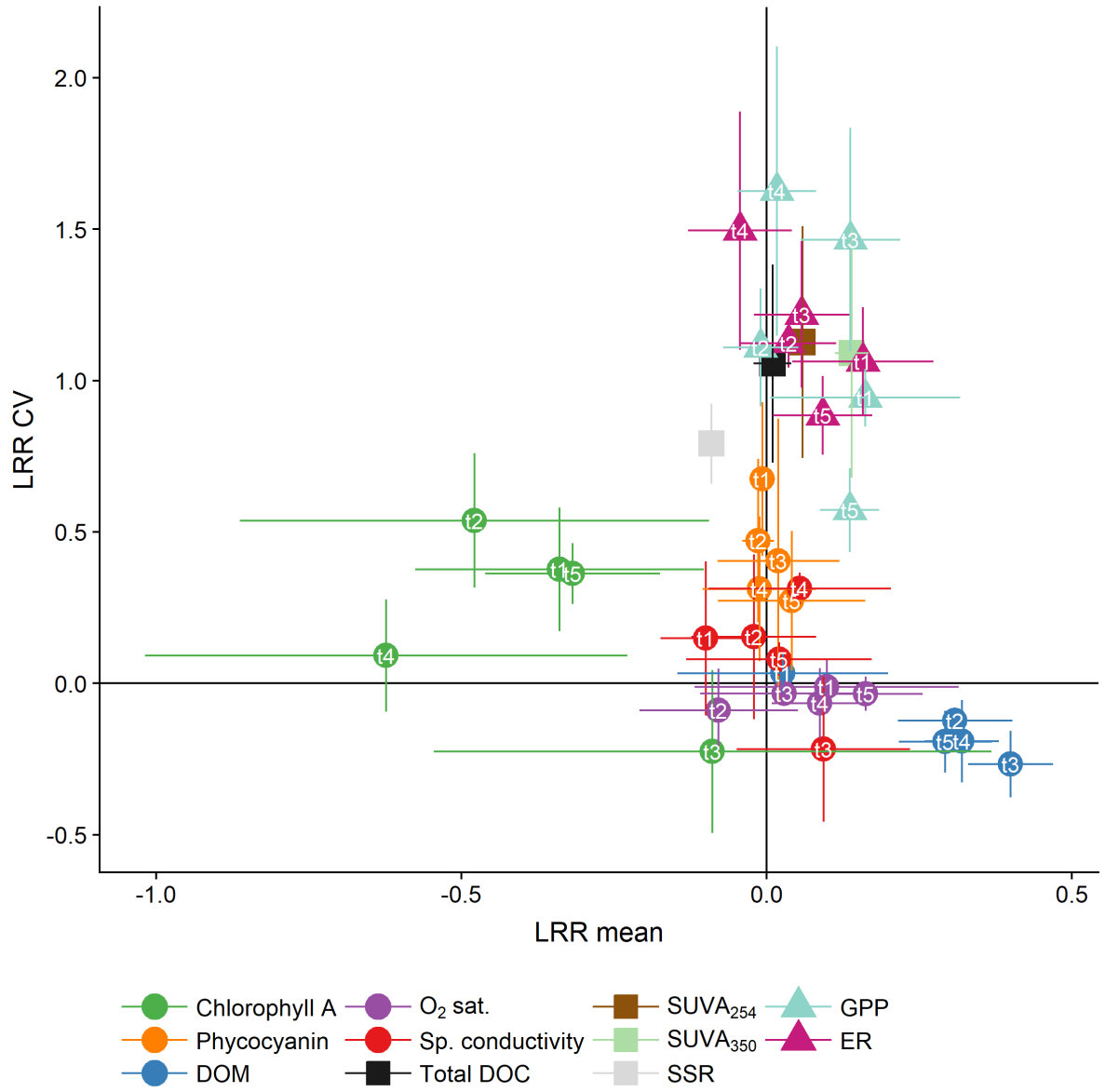
784


A
Nutrients
M
P
Light
B
Macrohyte biomass
Phytoplankton biomass
macrophytes and phytoplankton
λ1 = -0.0061
only phytoplankton
λ2 = -0.016
P'=0
M'=0
C
phytoplankton biomass
Time
Macrophytes
No Macrophytes
D
coefficient
of variation
M+
M-

785

786 5. A simple model of competition for light and nutrients between macrophytes and
787 phytoplankton (for details see Supplement). A: Schematic of interactions between
788 macrophytes (M) and phytoplankton (P). Macrophytes consume nutrients, which has a
789 negative indirect effect on phytoplankton. If phytoplankton biomass becomes too high, it
790 reduces light levels such that there is a negative indirect effect on macrophytes. Thus,
791 macrophytes are more strongly limited by light, and phytoplankton by nutrients. B: Zero-
792 growth curves of macrophytes (green line) and phytoplankton (blue line). Black points mark
793 the 2 alternative stable equilibria of either a macrophyte-and-phytoplankton state or an only-
794 phytoplankton state. Although these two states exist for the same level of nutrients in the
795 water, their stability (measured as the dominant eigenvalue lambda) differs: the only-
796 phytoplankton is more stable than the macrophyte-and-phytoplankton state. C: Simulated
797 time series of phytoplankton biomass in the presence (green) and in the absence (blue – note
798 second y-axis) of macrophytes for the same level of nutrients in the water. D: Coefficient of
799 variation of phytoplankton biomass estimated from 200 simulated sets.
800

801



802

803 6. Average log response ratios (LRR) for macrophyte presence on mean and CV. Effect sizes
804 were calculated differently for each data type: high frequency (●), metabolism (▲), or DOC
805 point measurements (■) – for details refer to the methods section. Each point shows the
806 average (mean ± se) macrophyte LRR across all tank pairs (N=4, Fig. 1) and in all
807 measurement periods (t1-t5, except for the DOC point measurements, where all 10
808 measurements were used to calculate LRR for mean and CV).

Tables

1. Parameters measured in high frequency using autonomous sondes. Prior to the experiment we performed a cross-comparison trial with all four sondes, after which we corrected all sensors for relative differences among them (i.e., “cross” = calibrated against each other). Chlorophyll-a sensors were additionally calibrated with samples taken during this trial that were analyzed for their chlorophyll-a content with high pressure liquid chromatography (HPLC). Oxygen sensors were calibrated against water-saturated air. (*fDOM-sensors measure emission at 365±5 and excitation at 480±40 nm. **For metabolism modelling mg/L output was used.)

Parameter	**Unit**	**Sensor type**	**Calibration**
Chlorophyll A	mg/L	Optical, fluorescence	HPLC, cross
Phycocyanin	Raw fluorescence	Optical, fluorescence	cross
fDOM	Raw fluorescence	Optical, fluorescence *	cross
Dissolved oxygen	% saturation**	Optical, luminescence	Saturated air, cross
Conductivity	µS/cm	4-electrode cell	Conductivity standard
Temperature	°C	Thermistor	cross

2. Statistical results of GAM-models testing time series of water parameters and metabolic rates. Results are from individual models (one model per parameter and measurement period). For mean and CV of water parameters, N per model is 768 for t1-t3 and 480 for t4 and t5. For metabolic rates, N per model is 8 23for t1-t3 and 5 for t4 and t5. Trends ($p<0.1$) indicated by bold font, significant results ($p<0.05$) indicated by underlined bold font. t-value = model estimate / model estimate SD, Rsq = R squared of model fit.

	t1			t2			t3			t4			t5		
Mean	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq
Chlorophyll A	1.724	**0.085**	0.809	2.6961	**0.007**	0.945	0.355	0.722	0.863	3.140	**0.001**	0.916	3.600	**≤0.001**	0.927
Phycocyanin	0.311	0.756	0.748	0.637	0.524	0.752	-0.445	0.656	0.865	0.006	0.995	0.883	-0.727	0.467	0.875
fDOM	-0.302	0.762	0.641	-4.923	**≤0.001**	0.889	-9.620	**≤0.001**	0.963	-6.690	**≤0.001**	0.983	-6.553	**≤0.001**	0.966
Dissolved oxygen	-0.877	0.380	0.758	1.163	0.245	0.779	-0.350	0.726	0.816	-2.013	**0.044**	0.856	-3.265	**0.001**	0.892
Temperature	-0.082	0.934	0.448	0.386	0.699	0.734	-0.370	0.711	0.646	0.657	0.511	0.775	-0.113	0.910	0.901
Conductivity	2.064	**0.039**	0.968	0.112	0.911	0.939	-1.165	0.244	0.907	-0.533	0.594	0.875	-0.019	0.985	0.884
CV	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq
Chlorophyll A	-2.041	**0.041**	0.784	-3.310	**0.001**	0.799	1.578	0.115	0.551	-0.388	0.698	0.661	-2.805	**0.005**	0.734
Phycocyanin	-4.846	**≤0.001**	0.668	-2.092	**0.037**	0.557	-1.354	0.176	0.621	-2.105	**0.035**	0.541	-1.886	**0.059**	0.696
fDOM	-0.052	0.958	0.508	1.119	0.263	0.357	4.036	**≤0.001**	0.426	0.746	0.456	0.629	0.431	0.666	0.492
Dissolved oxygen	0.244	0.808	0.617	1.186	0.236	0.558	0.949	0.343	0.319	0.566	0.571	0.363	0.312	0.755	0.404
Temperature	-0.235	0.816	0.524	-0.253	0.801	0.446	0.914	0.361	0.257	0.193	0.847	0.415	0.886	0.376	0.430
Conductivity	-0.278	0.781	0.339	-0.966	0.334	0.358	1.664	**0.096**	0.374	-0.989	0.323	0.464	-0.062	0.950	0.583
Metabolism	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq
P	-3.653	**≤0.001**	0.705	-1.165	0.249	0.461	-2.147	**0.036**	0.169	1.381	0.176	0.046	-3.395	**0.002**	0.406
R	-3.470	**0.001**	0.329	0.121	0.905	0.545	-0.367	0.360	0.456	-0.415	0.681	0.235	-0.346	0.340	0.230
P:R	0.160	0.874	-0.033	1.816	**0.074**	-0.005	-4.812	**≤0.001**	0.090	-3.389	**0.002**	0.303	-0.650	0.520	0.119